STUDIES ON THE INHIBITORY EFFECTS OF SUGARS UPON PLANT ROOTS

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GÖRAN STENLID

UPPSALA 1959

ALMQVIST & WIKSELLS BOKTRYCKERI AB

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INAUGURAL DISSERTATION

BY

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BY DUE PERMISSION OF THE FACULTY OF SCIENCE
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THE BOTANICAL AUDITORIUM, MAY 13, 1959, AT 10 A.M.
FOR THE DEGREE OF DOCTOR OF PHILOSOPHY.
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Introduction

The present paper is in the main a summary of the following communications published in Physiologia Plantarum by the author:

I. Toxic effects of D-mannose, 2-desoxy-D-glucose and D-glucosamine upon respiration and ion absorption in wheat roots. — Physiol. Plant. 7: 173–181 (1954).
II. A comparison of the toxic effects of some sugars upon growth and chloride accumulation in young wheat roots. — Physiol. Plant. 10: 807–823 (1957).
III. Some differences between the accumulation of chloride and nitrate ions in excised wheat roots. — Physiol. Plant. 10: 922–936 (1957).
IV. On the occurrence of surface-located β-glucosidase and β-galactosidase activities in plant roots. — Physiol. Plant. 10: 1001–1008 (1957).
V. On the effect of some sugars and of 2,4-dinitrophenol upon the absorption of phosphate ions by excised roots. — Physiol. Plant. 12: 199–217 (1959).
VI. Species differences between plant roots in the reaction to inhibitory sugars. — Physiol. Plant. 12: 218–235 (1959).
VII. Inhibitory effects of 2-desoxy-D-galactose upon plant roots. — Physiol. Plant. 12: 310–313 (1959).

In the following, these papers will be referred to by the Roman numerals I–VII.

An important method for the study of metabolism is the application of antimetabolites and antagonists (for reviews see Woolley 1952, Matthews 1958). The purpose of the present investigation was to use some sugars with inhibitory action as tools when studying the metabolism of plant roots. Sugars structurally related to glucose have been applied and the inhibition caused by them has been reversed by addition of other sugars.

The pioneer work in this field was performed by L. Knudson (1915–1917) who showed that mannose and galactose are toxic to roots of several plants and that it is possible to relieve the toxicity by additions of glucose and sucrose.

With the development of culture methods for excised roots and plant tissues, the interest in the suitability of various sugars as a carbon source for such objects increased. The investigations were mainly concentrated on the sugars which produce a good growth, and as a rule the

effects of sugars unsuitable as a carbon source were not examined in detail (for references see I, II, VI). During the last years, however, STREET and co-workers in extensive studies have investigated the effects of inhibitory sugars upon excised tomato roots (see FERGUSON *et al.* 1958 a, b, MORGAN and STREET 1959). In the present investigation some processes in plant roots, which can be expected to respond to alterations in the carbohydrate metabolism, were selected for a closer examination, namely growth, respiration and ion absorption.

Carbohydrates participate more or less directly in most of the reactions in plant roots but the main part of their functions can be grouped into two different categories:

1) Carbohydrates are, through the respiratory metabolism, the principal source of energy for growth and for absorption and transport of different substances.

2) Carbohydrates are necessary as building units in the production of new root substance. This is evident in the first place for substances of the polysaccharide type (cellulose, hemicelluloses, pectic substances) but also for many other substances. The protein synthesis should be especially mentioned, as sugars are necessary for the production of acceptors of the reduction products of nitrate.

Obviously the most complicated effects can be expected upon growth, being the integrated result of numerous processes. In growth, sugars are of prime importance both as building units and as an energy source. In respiration and ion absorption, on the other hand, the sugars are mainly used as a source of energy.

Roots from young seedlings grown on moist filter papers have been used in most of the experiments. The experiments on oxygen uptake and chloride absorption were performed with excised roots, as a rule during a period of 4 hours. With this short duration of the experiments secondary effects from growth inhibitions and also complications from possible bacterial contaminations were reduced. Oxygen uptake was determined with a Warburg-Barcroft apparatus, ion absorption through analyses of the external solution. In the experiments on ion absorption dilute solutions (about 10^{-3} *M*) were used, and the active accumulation predominated. In the growth experiments intact seedlings were used and the root elongation was measured after a growth period of 24 hours.

The following abbreviations will be used: 2-DG (2-desoxy-D-glucose), 2-DGal (2-desoxy-D-galactose), DNP (2,4-dinitrophenol), and ATP (adenosinetriphosphate). When the sugar names are used without prefix the D-forms are meant.

Oxygen uptake

In freshly excised roots, mannose, glucosamine and 2-DG inhibit oxygen uptake (I, VI). The inhibition is distinct, but does not amount to more than 30 to 40 per cent even in 0.05 *M* solutions. Galactose and 2-DGal do not inhibit oxygen uptake (I, VI, VII). If the control solution contained glucose or galactose (0.01 *M*) no or only a very slight inhibition was obtained with the inhibitory sugars.

In excised roots which have been pretreated in aerated distilled water the oxygen absorption of wheat and cucumber roots is enhanced by additions of glucose, galactose, mannose and 2-DG (VI). The nature of this stimulation is not clear, and further investigations are necessary. It is possible that the stimulation is of different kind for different sugars as the respiratory increase caused by glucose and galactose is combined with stimulation of chloride absorption in contrast to what is found for mannose and 2-DG.

In a recent paper Morgan and Street (1959) report that mannose inhibits the utilization both of other sugars and of pyruvate as respiratory substrates in excised tomato roots. They found a lowered respiratory quotient in the presence of mannose, which seems to suggest that respiration in this case is not equivalent to the respiration with sucrose, glucose or galactose as a substrate. Probably some non-carbohydrate substance is utilized. They also conclude that the utilization of externally added sugars is affected by mannose in another manner than is the endogenous respiration.

A possible explanation of the results on oxygen uptake is that the inhibition is connected with the activity of hexokinase, which takes part in the initial phase of glycolysis. All the sugars inhibitory to respiration (mannose, 2-DG and glucosamine) are acted upon by hexokinase, whereas galactose and 2-DGal are not (cp. II, VII). An unmodified glucose structure at carbon atoms 3, 4, 5 and 6 seems to be a necessary requirement for the hexokinase substrates (see Dixon and Webb 1958, p. 288). The inhibition may either be due to an effect upon hexokinase itself or perhaps more likely to effects produced by some substance formed more or less directly through the activity of hexokinase. 2-DG-6-phosphate *e.g.* inhibits both phosphoglucoisomerase and phosphoglucomutase (Wick *et al.* 1957, Kipnis and Cori 1959). In addition accumulation of glucose-6-phosphate may secondarily inhibit hexokinase (Crane and Sols 1954). According to Morgan and Street (1959) externally applied mannose-6-phosphate is not inhibitory to tomato roots, which may be due to slow penetration, however.

Ion absorption (I, II, III, V, VI, VII)

The uptake of three different anions has been studied, *viz.* chloride, nitrate and phosphate ions. In the absorption of chloride ions, sugars are probably utilized only in a non-specific way as a source of energy. The absorption of nitrate and phosphate ions, on the other hand, can be assumed to require sugars also for more special purposes.

The effect of sugars was studied in two different types of roots, namely freshly excised roots and excised roots which had been pretreated in continuously aerated water before the experiments (III). Initially it was expected that pretreated roots, according to their lower carbohydrate content, should give a slower ion absorption from the control solutions. It turned out, however, that in addition to a decrease in carbohydrate content other effects were obtained which complicated the situation. The absorption of chloride ions from the control solutions without sugars was strongly increased after pretreatment in distilled water. This effect upon chloride absorption was observed with all species tested (III, VI). The absorption of phosphate and nitrate ions in wheat roots was decreased by a similar pretreatment (III, V). Other species have not been investigated in detail with respect to these two ions, but it seems as if the decrease of absorption is much less pronounced in cucumber roots than in wheat roots. In any case, a strongly enhanced absorption after the pretreatment has only been found for chloride ions.

Mannose and 2-DG produced inhibitions of *chloride uptake* in all species investigated. Glucosamine and 2-DGal, which were only tested with wheat roots, likewise inhibited chloride uptake. Also in pretreated roots mannose and 2-DG caused a decreased chloride absorption as compared to the control. As oxygen uptake was increased by mannose in pretreated roots it may be assumed that the respiratory stimulation caused by mannose is not utilized for the absorption of chloride ions.

The effects of glucose and galactose are rather weak in freshly excised roots. As a rule these sugars stimulate chloride absorption in pretreated roots. Only in pea roots a distinct inhibition by galactose was observed.

The inhibitory effects of sugars upon chloride absorption were found to be relieved by glucose and galactose in all cases that have been investigated (except for pea roots where galactose itself is inhibitory).

A quantitative analysis of the inhibition in various combinations of inhibitory sugars with glucose and galactose showed that in wheat roots a competitive antagonism probably exists between the inhibitory sugar

and the externally added relieving sugar (II). The significance of this antagonism is discussed below (see pp. 7–10).

It was tentatively suggested above that the inhibition of oxygen uptake is in some way connected with the activity of hexokinase. As regards chloride absorption the agreement is not equally apparent, but it can be noted that galactose gives no and 2-DGal only weak inhibition of chloride uptake in wheat roots, whereas the other inhibitory sugars give fairly strong inhibitions.

Nitrate absorption is affected by sugars much in the same way as is chloride absorption. In wheat roots, mannose, 2-DG and 2-DGal thus inhibit nitrate absorption, and glucose stimulates it. Galactose is weakly inhibiting in freshly excised roots, but stimulates nitrate absorption in pretreated roots (III, VI).

Phosphate absorption in pretreated wheat roots is stimulated by different sugars (glucose, mannose, galactose, 2-DG, sucrose). In freshly excised roots weak inhibitions were obtained with mannose. Galactose is the most effective sugar in stimulating phosphate absorption in wheat roots (V).

There are thus obvious differences between the absorption of the three ions as affected by different sugars. The decrease of the absorption capacity for nitrate and phosphate in pretreated roots is probably related to a greater requirement of carbohydrates in the absorption of these ions than in the absorption of chloride ions. Nitrate and phosphate ions take part in the root metabolism and the absorption has a close relationship to the assimilation.

In some cases it seems as if galactose (nitrate absorption, III) or mannose (phosphate absorption, V) can replace the natural substrate although they are utilized at a lower efficiency. In freshly excised roots a slight inhibition is obtained, which may be explained as due to displacement of the natural substrate. In pretreated roots with lower sugar content, where the competition with the natural substrate is less important, positive effects are obtained.

Phosphate absorption is obviously more easily stimulated by sugars than is the absorption of chloride and nitrate ions. Even mannose and 2-DG thus cause positive effects in pretreated roots. A study of the literature dealing with phosphate absorption in plant roots reveals that probably some special dependence upon the oxidative phosphorylation exists for phosphate absorption. The effect of 2,4-dinitrophenol (DNP) upon the absorption of phosphate and chloride was therefore determined by taking samples simultaneously from solutions containing both these ions. Low concentrations of DNP (10^{-6} M — 10^{-5} M) sti-

TABLE 1. *Effect of various substances and of pretreatment of the roots with distilled water upon some processes in plant roots. + = stimulation, (+) = weak stimulation, − = inhibition, 0 = no distinct effect.*

		Substance (Treatment) applied							
Roots used	Process studied	Pretreatm. aq. dest.	Gal-actose	Man-nose	Glu-cose	2-DG	2-DGal	DNP 10^{-5} *M*	Salicyl-acid 10^{-4} *M*
Wheat, freshly exc.	Cl-absorption	+	(+)	−	0	−	−	+	+
"	NO_3-absorption	−	−	−	+	−	−	−	−
"	Phosphate abs.	−	+	−	0			−	−
"	Oxygen uptake	−	(+)	−	+	−	0	+	0
Wheat, exc., pretr. 48 h.	Cl-absorption		+	−	+	−			0
"	NO_3-absorption		+	−	+	−			
"	Phosphate abs.		+	+	+	+	(+)		
"	Oxygen uptake		+	+	+	+			0
Wheat, seedl.	Growth		−	−	0	−	−	0	+
Cucumber seedl.	"		(+)	−	(+)	−	−		
Cress, seedl.	"		(+)	−	(+)	−	−		

mulated chloride absorption but inhibited phosphate absorption (V). Similar results were obtained with salicylic acid (III, V), which is likewise known to interfere with oxidative phosphorylation. Nitrate absorption is affected by DNP and salicylic acid mainly in the same way as is phosphate absorption. In higher concentrations DNP and salicylic acid inhibit the absorption of all three ions. The effects of some substances upon ion absorption in wheat roots are summarized in Table 1.

Phosphate and nitrate absorption are thus both inhibited by concentrations of DNP and salicylic acid which stimulate chloride uptake. Phosphate and nitrate ions differ in their reaction to mannose and 2-DG, which stimulate phosphate absorption in pretreated roots, but strongly inhibit nitrate absorption. As an explanation it is suggested that the absorption of phosphate and nitrate is dependent upon oxidative phosphorylation in a more direct way than is chloride absorption. In addition it is assumed that the absorption of phosphate and nitrate is stimulated by the presence of special organic substances. If these substances are supposed to be sugars in the case of phosphate absorption and some intermediate of the carbohydrate degradation in the case

of nitrate absorption the different behaviour of the two ions towards 2-DG and mannose may be explained. The appropriate acceptors in nitrate assimilation should be formed only from glucose and galactose and not from mannose, 2-DG and 2-DGal.

The results in the present investigation as well as recent results with labelled phosphate (Loughman and Russell 1957, Miettinen and Savioja 1958) support the hypothesis that phosphate is first incorporated in ATP through oxidative phosphorylation and thereupon transferred to sugar phosphates by enzymes of the hexokinase type.

Root growth. The nature of the sugar antagonism

The growth of seedling roots has been determined after a growth period of 24 hours (in some few experiments 18 hours) in nutrient solutions (II, III, V, VI, VII).

Mannose, 2-desoxy-D-glucose (2-DG) and 2-desoxy-D-galactose (2-DGal) were found inhibitory to all species tested (II, VI, VII). Wheat root growth was inhibited by glucosamine, which was only tested with this species. 50 per cent inhibition of wheat root growth was obtained with 10^{-3} *M* glucosamine, $3 \cdot 10^{-4}$ *M* galactose, $3 \cdot 10^{-4}$ *M* mannose, $6 \cdot 10^{-5}$ *M* 2-DGal, and $3 \cdot 10^{-5}$ *M* 2-DG. Galactose in concentrations of 10^{-4} *M* —10^{-3} *M* caused distinct inhibitions in several species (oats, barley, wheat, rye), whereas other species (cucumber and cress) were not inhibited even by 10^{-2} *M* galactose (II, VI). There are also intermediate species with respect to galactose (maize and flax), where a slight inhibition is obtained in 10^{-2} *M* solutions (VI).

The inhibitory effects are reversed by additions of glucose (II, VI, VII), which as a rule in itself produces only slight effects upon root growth. As in the case of chloride absorption the data seem to agree with the hypothesis of a competitive antagonism between the externally added sugars (II).

In cress and cucumber roots, where the growth is not decreased by galactose, the inhibitions caused by mannose, 2-DG and 2-DGal are reversed also by galactose (VI, VII). Galactose thus seems to act similarly as glucose as an antagonist to mannose and 2-DG in these roots.

The antagonistic effects of sugars imply several complicating factors. Firstly, it should be emphasized that if it is supposed that the inhibitory effect of a sugar is exerted at a special site in the growth mechanism (or the respiratory or ion absorbing system) it is not necessary to assume that the reversing antagonism is manifested at the same site. It is pos-

sible that the reversal is due to antagonism during the absorption or the transport to the crucial site. In this way the concentration of the inhibiting sugar may be so much decreased that a weakened inhibition is obtained. If the sugar added is transformed to some other compound, which is the real antagonist, the reversal may be exerted during this transformation. That an inhibition caused by *e.g.* galactose is competitively reversed by externally added glucose, does therefore not prove that the inhibitory action is of the competitive type or that it is due to antagonism against glucose. The inhibitor may be of a non-competitive type even if it is competitively antagonized by a reversing substance. The real nature of the inhibitory action of the sugars applied therefore still remains unknown, even if it seems plausible to assume some sort of competition with glucose as the cause of the inhibition.

That an impaired transport may lead to a decreased inhibition may be concluded from the experiments with dinitrophenol, which was shown to moderate the inhibitory effects of galactose and mannose to some extent (V). The direct data on the absorption of the different components in sugar mixtures do not allow any definite conclusions about the extent of the antagonism exerted in the absorption (see VI). The low efficiency of the endogenous sugar supply as compared to externally added glucose (II, VI) supports the view that the reversing effect is due to an antagonism at some point in the absorption or transport of the inhibitory sugars, or at some surface-located part of the growth mechanism. A great part of the endogenous sugar can be assumed to be located in the vacuoles and it is difficult to estimate to what extent it is available for different processes. Another uncertain factor is that the sugars may be phosphorylated during the absorption and that the sugar phosphates formed are of special significance in growth and other processes.

A possibility which should be seriously considered is that galactose may be converted to glucose in the roots (II, VI). The uridine phosphate sugars and the enzymes responsible for this interconversion have been demonstrated in plant tissues, but it is uncertain if the results are applicable to the present investigation with roots.

Antagonistic effects may be obtained both with substances which are utilized by the roots and with indifferent substances as well as with substances which are inhibitory themselves. If two inhibitory sugars with widely different activities are added together, the less inhibitory sugar may reverse the inhibition caused by the more inhibitory one (experiments with galactose and 2-DGal in flax roots, VII). This can be explained on the assumption that the two sugars compete for the same sites.

In growth experiments with wheat roots, mannose and galactose are about equally effective inhibitors. If the two sugars are added together, the inhibition of the mixture is less than of a solution containing only one of the components, provided that the concentrations are kept within a certain range. Galactose thus stimulates root growth in the presence of mannose, and mannose stimulates root growth in the presence of galactose (VI). The results may theoretically be explained as caused by antagonism in two different absorption mechanisms. In view of the different effects of the two sugars upon respiration and ion absorption, however, it seems probable that there is some other difference also regarding their effect upon growth. The antagonism of galactose against mannose in the growth of wheat roots may be of the same type as in the chloride absorption of wheat roots or as in the growth of cress and cucumber roots. Mannose in its turn may be without inhibitory effects *e.g.* in cellulose synthesis and antagonize galactose there (cp. the experiments with Avena coleoptiles described by Ordin and Bonner 1957 and by Thimann *et al.* 1958). Ferguson *et al.* (1958 b), who obtained a mutual antagonism in experiments with excised tomato roots, likewise interpret their data as due to different types of inhibition resulting from different sugars.

Summarizing the inhibitory effects of the sugars studied, it is obvious that mannose, 2-DG and glucosamine form one group differing from galactose in several respects. Galactose is more specific in its action, as a rule inhibiting neither ion absorption nor respiration. Moreover, growth inhibition with galactose has not been observed in all species. 2-DGal is intermediate in its action between galactose and the other sugars.

As regards mannose, the inhibition of respiration may lead to a decreased ion absorption. We have still a rather incomplete view of the way in which the respiratory energy is transferred and exploited in the accumulatory mechanisms, and especially the role of the "high energy phosphates" is far from clear. It is therefore impossible to decide how the inhibition is caused, and it can only be stated that the part of the respiration taking place in the presence of mannose is poorly utilized for ion accumulation. Also the growth inhibition caused by mannose may be due to disturbed respiration. It is, however, possible that mannose acts as a structural antagonist towards glucose as a building unit or that the effect of mannose is complex, involving different components. What has been said about mannose is in the main valid also for 2-DG and glucosamine, although their action may deviate to some extent from that of mannose.

For galactose it must be supposed that it can replace glucose in some activities in the root but not in others. There are differences both between different processes in the same species and between the same process in different species. The difference between growth and respiration in wheat roots can be explained on the assumption that galactose is a satisfactory energy source for respiration and ion absorption, but that it is unsuitable for the synthesis of cellulose or some other fundamental structural substance. Galactose may be thought either to prevent the incorporation of some natural compound or it may itself be incorporated into polysaccharide units, which are biologically ineffective.

The differences between species in their reaction to galactose may be due to its conversion to glucose in those species where growth is not inhibited, but other explanations are possible as well. 2-DGal may be assumed to inhibit growth in the same manner as galactose. If this sugar cannot be converted to glucose, the inhibitory effects upon cress and cucumber roots are understandable.

As for the biochemical background to the inhibitions, it is impossible to give any definite suggestions, as the fate of different sugars in root metabolism is rather incompletely known. The sugars added may be inhibitory as such, displacing natural substrates from some enzyme. It is also conceivable that the sugars are converted to other compounds before their effect is exerted (cp. the inhibitory effect of 2-DG-6-phosphate mentioned on p. 3). In this way complicated inhibitory effects may arise, and in addition the reversing effects may be compound.

Matthews (1958) in a review of metabolite analogues points to the necessity of a close analysis of the effects before it is claimed that an antagonism is due to the action of the analogue in the form supplied to the organism. He stresses that likewise in many cases direct evidence is lacking for the suggestions that inhibitory effects are due to incorporation into larger molecules. Great caution is certainly justified in the case of sugars, where many metabolic pathways are possible, and it must be admitted that the present data do not permit more than tentative guesses about the mechanism of the inhibitory effects.

On the occurrence of β-glucosidase and β-galactosidase activities in plant roots (IV)

With *p*-nitrophenyl-β-D-glucoside, salicin and *o*-nitrophenyl-β-D-galactoside as substrates the occurrence of β-glucosidase and β-galactosidase activities was demonstrated in plant roots. The β-glucosidase activity was greater than the β-galactosidase activity.

Different sugars are known to inhibit β-glucosidase and in the present investigation *i.a.* 2-DG and mannose were shown to decrease the β-glucosidase activity in wheat roots. Gluconic acid lactone, which is a strong inhibitor of β-glucosidase, inhibited the β-glucosidase activity in wheat and cucumber roots. It had no distinct effect upon wheat root growth or chloride absorption, however, and it does not seem likely that β-glucosidase is of any significance in root growth or that the effect of inhibitory sugars upon growth is caused by an inhibition of β-glucosidase. No appreciable enzymatic activity was found in the solutions surrounding the roots. In view of the rapid inhibitory effect of the glucono-lactone it was concluded that the enzymatic activity responsible for the reactions studied was located rather superficially in the root. The inhibition with galactonic acid lactone of the cleavage of *o*-nitrophenyl-β-D-galactoside was taken as evidence for a similar β-galactosidase activity.

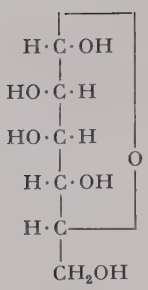
A hypothetical explanation of the inhibitory and reversing activities of sugars can be found in the transferring activities of glucosidases and galactosidases implying a coupling of sugars to each other. In this way inactive oligosaccharides might be formed from inhibitory sugars. On the other hand, new inhibitory sugars may also be synthesized through a similar mechanism. There is, however, no experimental evidence supporting such assumptions.

Correlations between structure and inhibitory effect

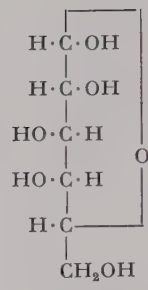
In this investigation inhibitory effects have been obtained with five sugars where the glucose structure is modified at carbon atom 2 or 4, but unchanged in the rest of the glucose molecule. All the tested sugars with these properties have produced inhibitions, whereas equally distinct inhibitions have not been observed with any other sugars. Formulae for glucose and the inhibitory sugars are given below.

Unfortunately, other aldohexoses are difficult to obtain and only D-gulose (unpublished experiments) and L-galactose (II) have been tested in some preliminary experiments, but none of these sugars produced any inhibitory effects.

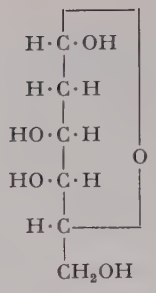
The ketohexoses D-fructose and L-sorbose are not inhibitory. D-fructose is a rather poor reversing substance towards the inhibitions caused by other sugars, much less effective than glucose and sucrose. In other investigations fructose (BRANNON 1923) was reported as inhibitory to roots, and sucrose was found toxic to excised wheat roots



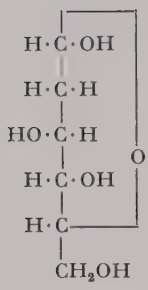
D-Mannose



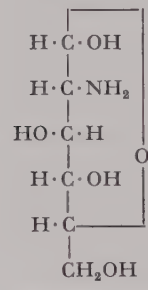
D-Galactose



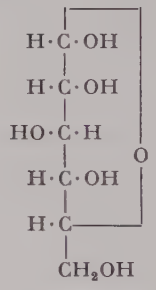
2-DGal
(2-desoxy-D-galactose,
2-desoxy-D-talose,
2-desoxy-D-*lyxo*-hexose)



2-DG
(2-desoxy-D-glucose,
2-desoxy-D-mannose,
2-desoxy-D-*arabino*-hexose)



D-Glucosamine



D-Glucose

(Burström 1941). These inhibitions seem to be of a more restricted and specific occurrence than those in the present investigation and they will not be discussed here. In tests with the ketohexose D-tagatose (which can be said to correspond to D-galactose among the aldohexoses) distinct inhibitions were obtained of the growth of wheat, cress and cucumber roots (unpublished experiments). These inhibitions were not reversed by glucose or galactose in the same way as the other inhibitions caused by sugars. It seems therefore possible that the inhibitions are due to some impurity in the preparation and not to the deviation in the steric structure of the sugar.

Substances with more profound modifications in the structure are as a rule indifferent, *i.e.* they neither inhibit nor reverse an inhibition (I). To this group belong sugar alcohols (*e.g.* D-mannitol corresponding to D-mannose and dulcitol corresponding to D- and L-galactose) and glycosides (*e.g.* α-methyl-D-mannoside and β-methyl-D-galactoside). The pentoses are either indifferent or in some cases (D-xylose and pos-

sibly L-arabinose) slightly relieving. In other investigations D-xylose has been found inhibitory in some cases (cp. below) but in the present study no inhibitory effects have been observed.

Notes on the inhibitory effects of sugars upon different organisms

Considering the almost universal occurrence of the principal reactions in glycolysis and the possible effects upon such reactions by inhibitory sugars, it may be of interest to know to what extent inhibitory effects of sugars have been observed in other organs than plant roots. In view of the tremendous literature dealing with the physiological effects of sugars, it is only possible to give some examples which seem of special interest in connection with the present investigation. For more extensive reviews see *e.g.* STEPHENSON 1949 (bacteria), LILLY and BARNETT 1953 (fungi) and PIGMAN 1957 (animal tissues).

Galactose. Different fungi, including yeast, are able to utilize galactose, although it is often less suitable than glucose as a carbon source. In some cases abnormal growth or other deviations have been observed (see HORR 1936). The metabolism of galactose has been studied in detail in connection with investigations on inducible (adaptive) enzymes. Many algae are able to utilize galactose, for the most at a lower rate than glucose (BRISTOL ROACH 1926, 1927, BURGE and WILLIAMS 1927). ROBBINS (1918) found that the moss *Ceratodon purpureus* can utilize galactose.

In leaves both galactose and mannose seem to be utilized, at least to some extent (for references see II and VI).

Normally galactose seems to be metabolized by animals. In some cases it may induce cataract or disturbed carbohydrate metabolism.

For *mannose* fewer data are available than for galactose. Most fungi, including yeast, seem to grow quite well with mannose as a source of carbon. Some algae can also utilize mannose (BRISTOL ROACH 1927). It gives rise to glycogen formation in different animals, in most cases probably after conversion to glucose. For bees, however, mannose is definitely toxic in contrast to all other sugars tested (see VOGEL 1931).

D-*xylose* as L-*arabinose* is utilized by many fungi. D-xylose has been reported as inhibitory to the growth of excised tomato roots (FERGUSON *et al.* 1958 b; this inhibition is not reversed by glucose) and to photosynthesis in *Chlorella* (HASSALL 1958). It is also unsuitable as a carbon source for *Scenedesmus* (BRISTOL ROACH 1926). In this connection it

can be mentioned that Brown *et al.* (1949) have suggested D-xylo-ketose as a growth stimulating factor in roots. In animals xylose may cause cataract in the same way as galactose.

2-desoxy-D-glucose, which in contrast to the other sugars mentioned above does not occur in nature seems to be inhibitory to all tissues where it has been tested (see Wick *et al.* 1957).

There are thus no quite general toxic effects of the inhibitory sugars (perhaps except for 2-DG). Mannose and galactose seem to be utilized by widely different organisms, at least to some extent. This might be taken as evidence against the inhibition of some fundamental enzyme as the cause of the inhibitory effects frequently observed. Quite as in plant roots limitations in permeability and transport together with transformations of the sugars complicate the interpretation in other organisms, however. It is further conceivable that only organs where the inhibited enzymes are rate-limiting respond to the inhibitory sugars. The non-toxicity of the sugars in many tissues can therefore scarcely be regarded as a proof that the inhibition does not imply effects upon some fundamental enzyme system.

Galactose and mannose are frequently found in glycosides and polysaccharides in plants. It could seem natural to suppose that a sugar occurring in a plant should not be inhibitory to the same plant, but it is doubtful if such an assumption holds good. Firstly, the inhibitory sugars very rarely occur in the free form in plant organs and it must be supposed that the formation of polysaccharides containing galactose and mannose proceeds without the formation of any considerable amounts of the free sugars as intermediates. Secondly, the rather specific action of galactose means that it is inhibitory only at some sites regulating growth, whereas galactose at other places in the plant may be harmless. Nevertheless, some interesting analogies may be found, which seem worth further investigation. Many orchid tubers contain an abundance of mannose in mannanes, and according to Wynd (1933) orchid seedlings readily use mannose as a carbon source. Cress seed mucilage contains galactose, and cress roots are not inhibited by D-galactose. Boas and Merkenschlager (1923) state that lupine seedlings germinate well in the presence of galactose, and they point out that the seeds contain abundant amounts of arabogalactan. On the other hand, many cases are found where such a parallelism does not exist, and it is certainly not possible to find any general relationship between content of a sugar and sensitivity to the same sugar.

It should finally be emphasized, that it is often difficult to decide if a sugar is inhibitory to a plant species, as different processes are

affected in different ways, and the state of development and the experimental methods play an important role. For the same reason a comparison between species regarding the sensitivity to inhibitory sugars is a rather intricate question.

Acknowledgements

The investigations summarized in this paper were carried out at the Institute of Plant Physiology, Royal Agricultural College, Uppsala, during the years 1952–1959. To Professor Henrik Lundegårdh, the former head of the Institute, who introduced me into experimental plant physiology, I express my sincere gratitude for his encouraging interest and most valuable support. I am very much indebted to Professor Börje Åberg, the present head, for valuable advice and many stimulating discussions throughout this work. The investigation has been greatly faciliated by the resources of the Institute, which have generously been put at my disposal. Cordial thanks are expressed to my colleagues at the Institute, expecially Dr Ivar Ekdahl, who has been of great help in various respects, and Dr Hemming Virgin.

To Professor Nils Fries, Uppsala, and to my teachers of botany at the University of Stockholm, Professor Gottfrid Stålfelt, Professor Folke Fagerlind, and the late Professor Otto Rosenberg, I owe deep thanks for stimulating teaching and encouragement.

The investigation has been supported by grants from Magnus Bergvalls Stiftelse and the Swedish Natural Science Research Council.

Institute of Plant Physiology, Royal Agricultural College, Uppsala 7, March 1959.

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